THE $^{13}C/^{12}C$ RATIO IN HONEY

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Summary

Values of the ratio ^{13}C to ^{12}C are given for 84 representative honeys from the United States and 35 honeys imported into the United States. Remarkably uniform results were obtained; the average $\delta^{13}C$ for all samples was $-25{\cdot}4\%$, and the coefficient of variation only $3{\cdot}86\%$.

The values are those expected from plants with the C_3 (Calvin) photosynthetic pathway. They provide the basis for the detection of corn-derived syrups in honey.

Introduction

In the course of a study to find ways to detect the illegal (undeclared) admixture of high-fructose corn syrup with honey, we investigated the applicability of the $^{13}C/^{12}C$ ratio to this problem. Atmospheric CO_2 contains both of the stable carbon isotopes of atomic weight 12 and 13. Referred to a standard limestone, the relative concentration of the ^{13}C isotope ($\delta^{13}C$) is $-7^{\circ}/_{oo}$. Photosynthetic fixation of atmospheric CO_2 has a pronounced isotopic effect; subsequent biochemical reactions scarcely discriminate, so the isotopic ratio established by the photosynthetic mechanism remains essentially unchanged and is characteristic of the photosynthetic mechanism. It has been shown (Bender, 1971; Smith & Epstein, 1971) that the ratio differs considerably among plants, two groups of values being observed. Plants using the Hatch-Slack C_4 carboxylic acid pathway in CO_2 fixation (Hatch et al., 1967) have values of $\delta^{13}C$ in the range $-10^{\circ}/_{oo}$ to $-20^{\circ}/_{oo}$, and those with only the Calvin C_3 cycle (Calvin & Bassham, 1962) show values of $-22^{\circ}/_{oo}$ to $-33^{\circ}/_{oo}$. Corn (*Zea mays*) is in the former group, and the few plants yielding nectar and honeydew that have previously been analysed are in the latter group, although in a rather wide range (Smith & Epstein, 1971). In order to evaluate this approach to the problem at hand, it was necessary to analyse a representative group of honey samples for $\delta^{13}C$ values. A summary report of these analyses (Doner & White, 1977), and a five-laboratory collaborative study of the use of this test for detecting adulteration of honey with corn products (White & Doner, 1978), appear elsewhere.

We now record the complete data summarized in the former report, for 84 samples of United States honey, together with corresponding data for 35 samples of honey from other countries.

Materials and Methods

Samples were selected from a collection of nearly 500, from the 1974-75 honey crops obtained by producers in the United States and certified by them as genuine honey. Selection criteria (Doner & White, 1977) were intended to ensure that the samples represented all commercially significant United States honeys, a variety of season mixtures from over the entire country, and samples of several different floral types from different areas.

Imported honeys were selected from a group of about 50 submitted by two importers and a domestic packer, to be representative of the majority of honey types imported into the USA. Selection was intended to eliminate duplication. Only limited information was obtained on the identities of the samples, and the time available did not permit authentication by their producers or by Government authority.

Carbon isotope ratios were determined by Geochron Laboratories Division of Krueger Enterprises Inc. (Cambridge, Mass.)* on an AEI MS-20 double-collecting, 180°-sector mass spectrometer with a dual-capillary inlet. Usual corrections were applied to the measured differences, including any zero enrichment in the capillary inlet system, valve mixing between sample and standard valves, tailing of major on to minor peak signals, and contribution of ^{17}O to the mass 45 signal. Samples were combusted in purified O_2 at about 850°C, and the gases were recirculated over CuO at 850°C for 10 minutes; water and CO_2 were frozen, excess O_2 was removed, and CO_2 transferred to a sample flask for analysis. The overall reproducibility for the system was stated to be 0·3 per mil in the ratio; however, five duplicate samples of honey agreed within 0·1 per mil. Interlaboratory crude oil reference samples also agreed within 0·1 per mil overall.

Results are expressed as $\delta^{13}C$ units:

$$\delta^{13}C^{\circ}/_{\circ\circ} = \left[\frac{^{13}C/^{12}C\ \text{sample}}{^{13}C/^{12}C\ \text{standard}} - 1\right] \times 10^{3}$$

Reproducibility, including sample combustion, CO_2 purification, and isotope ratio measurements, was stated to be $0{\cdot}3^{\circ}/_{\circ\circ}$, although duplication to $0{\cdot}1^{\circ}/_{\circ\circ}$ was common.

Results

Table 1 shows the values for $\delta^{13}C$ obtained on the United States honey samples, and Table 2 gives values for imported honeys. Table 3 summarizes the data, and provides estimates of their variability.

Discussion

The values obtained are remarkably uniform; in fact, the variability encountered here (overall coefficient of variability 3·86%) is the smallest seen in the literature for any constituent or property of honey.

This uniformity may arise in part from the fact that these measurements on honey largely represent the isotope ratio for the monosaccharides, with no significant contribution from other plant components. Different constituents (proteins, fats, carbohydrates) of a single plant sample are reported (Whelan et al., 1970) to vary in $\delta^{13}C$ rather more than is seen here for all samples.

It is tempting to examine the data for significant trends and differences, but the nature of the samples rules this out. Free-flying honeybees forage for miles around the colony, so none of these samples may properly be considered unifloral; furthermore, most of them have been taken from the combined output of many colonies. Differences in $\delta^{13}C$ among the various honeys listed should not be inferred from the data. In order to detect plant-specific differences the use of hand-collected nectars would be more appropriate. Isotope ratio analysis requires only milligram samples, and this would facilitate such research.

The relative uniformity found for all samples, together with the even smaller variability in $\delta^{13}C$ among samples of high fructose corn syrup (average ^{13}C for 4 samples from

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* Krueger Enterprises, Inc., Geochron Laboratories Division, 24 Blackstone Street, Cambridge, Massachusetts 02139. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

4 makers = −9·7‰, range = −9·5 to −9·8, s = 0·14, *c.v.* = 1·7%) allows the detection of the latter in honey; details on this application appear in another paper (White & Doner, 1978), which shows that the probability of an authentic honey having $\delta^{13}C$ less negative than −21·5‰ is 1 in 25 000.

After the completion of this manuscript it was learned of similar research, with comparable results, at the Institute for Botany and Microbiology of the Technical University of Munich by Zeigler et al. (1977).

TABLE 1. The $^{13}C/^{12}C$ ratios of selected United States honeys.

Beekeeper's estimate of floral type	*State*	$\delta^{13}C$ (‰)
Anacardiaceae		
Schinus molle (pepper tree)	Florida	−25·0
Aquifoliaceae		
Ilex glabra (gallberry)	Georgia	−25·6
Compositae		
Aster spp. (aster)	Ohio	−25·7
Carthamus tinctorius (safflower)	California	−25·2
Centauria maculosa (spotted knapweed)	Montana	−25·3
Centauria solstitialis (star thistle)	California	−26·3
Helianthus spp. (sunflower)	N. Dakota	−25·6
Solidago spp. (goldenrod)	Minnesota	−25·5
Cornaceae		
Nyssa ogeche (tupelo)	Florida	−26·0
Cyrillaceae		
Cyrilla parvifolia (titi)	Georgia	−24·2
Euphorbiaceae		
Sapium sebiferum (tallow tree)	Texas	−26·4
Labiatae		
Mentha spp. (mint)	Washington	−25·2
Monarda punctata (horsemint)	Texas	−26·5
Salvia spp. (sage)	California	−24·2
Thymus spp. (thyme)	New York	−26·3
Trichostema lanceolatum (blue curls)	California	−25·4
Leguminosae		
Acacia berlandieri (huajillo)	Texas	−24·9
Acacia greggii (catsclaw)	Arizona	−22·8
Glycine soja (soybean)	Iowa	−26·2
,,	Minnesota	−25·4
Lotus corniculatus (trefoil)	Iowa	−25·7
Medicago sativa (alfalfa)	Arizona	−26·1
,,	California	−24·8
,,	Colorado	−25·1
,,	Idaho	−24·8
,,	Montana	−25·3
,,	Nebraska	−25·8
,,	S. Dakota	−24·9
,,	Utah	−24·4
,,	Washington	−25·5
,,	Wyoming	−25·1
Melilotus spp. (sweetclover)	Kansas	−26·6
,,	N. Dakota	−26·2
Medicago + *Melilotus* (alfalfa + sweetclover)	Colorado	−24·6
,,	Iowa	−26·0
,,	Montana	−24·0
,,	Nebraska	−26·2
Phaseolus limensis (Lima bean)	California	−24·2
Prosopis glandulosa (algaroba)	Hawaii	−22·5

Beekeeper's estimate of floral type	State	$\delta^{13}C$(°/₀₀)
Prosopis glandulosa (mesquite)	Texas	−26·9
Robinia pseudoacacia (locust)	Tennessee	−24·8
Trifolium spp. (clover)	Arkansas	−26·1
,,	Illinois	−26·2
,,	Indiana	−25·7
,,	Michigan	−26·1
,,	Minnesota	−25·9
,,	Nebraska	−25·0
,,	New York	−24·7
,,	Ohio	−25·1
,,	Pennsylvania	−25·1
,,	S. Dakota	−25·7
,,	Wisconsin	−25·7
Vicia villosa (vetch)	Oregon	−25·3
Magnoliaceae		
Liriodendron tulipifera (tulip tree)	N. Carolina	−25·3
Malvaceae		
Gossypium hirsutum (cotton)	Oklahoma	−24·7
Onagraceae		
Epilobium angustifolium (fireweed)	Oregon	−25·4
Palmae		
Sabal spp. (palmetto)	Florida	−24·7
Polygonaceae		
Erigonum fasciculatum (wild buckwheat)	California	−24·1
Fagopyrum esculentum (buckwheat)	New York	−25·2
Rosaceae		
Rubus spp. (blackberry)	Washington	−26·1
Rutaceae		
Citrus spp. (orange)	California	−23·0
Citrus spp. (orange-grapefruit)	Florida	−23·8
,,	Florida	−23·5
Tamaricaceae		
Tamaris gallica (tamarisk)	New Mexico	−25·1
Tiliaceae		
Tilia americana (basswood)	Wisconsin	−25·6
Unclassified 'natural' season blends		
Northeast	New York	−26·0
,,	Pennsylvania	−25·5
Southeast	Florida	−25·5
,,	Mississippi	−24·7
East Central	Illinois	−25·9
,,	Michigan	−25·5
West Central	Iowa	−25·5
,,	Minnesota	−24·9
,,	Missouri	−25·1
Intermountain	Utah	−23·0
Southwest	Arizona	−22·7
,,	Louisiana	−27·4
,,	Texas	−24·5
West	California	−25·2
,,	Oregon	−25·4
Honeydew honey		
Polarization (°S)		
+ 14·5	California	−23·5
+ 5·0	N. Carolina	−25·6
+ 15·5	Utah	−23·7
+ 2·1	Virginia	−25·1

TABLE 2. $^{13}C/^{12}C$ ratios of honeys imported into the United States.

Class on Pfund grader	*Country*	$\delta^{13}C‰$
light amber	Brazil	−25·0
amber	Brazil	−25·6
	Mexico, Yucatan	−24·4
white	Mexico, Monterrey	−23·9
light amber	Mexico, Tampico	−25·8
extra light amber	Mexico, Oaxaca	−26·4
	Mexico, Guadalajara	−27·5
	Mexico, Morelos	−26·9
light amber	Colombia	−25·9
extra light amber	Argentina	−27·1
white	Argentina	−27·4
white	Argentina	−27·0
white	Argentina	−26·2
white	Venezuela	−27·4
white	Venezuela	−25·7
light amber	Venezuela	−25·8
light amber	Dominican Republic	−25·0
white	Chile	−26·8
pile 1	Chile	−26·0
extra light amber	Chile	−26·5
white	Costa Rica	−25·0
extra light amber	Uruguay	−25·9
extra light amber	Guatemala	−25·3
extra white	Canada	−26·0
light amber	Canada	−25·8
light amber	Chinese People's Republic	−26·6
dark amber	Chinese People's Republic	−26·6
light amber	Australia	−24·2
Beekeeper's estimate of floral type		
Boraginaceae		
Echium lycopsis (Salvation Jane)	Australia	−25·2
Cunoniaceae		
Weinmanni racemosa (Kamahi)	New Zealand	−24·8
Eucryphiaceae		
Eucryphia (leatherwood)	Tasmania	−24·5
Labiatae		
Thymus vulgaris (thyme)	New Zealand	−24·9
Leguminosae		
Trifolium repens (white clover)	New Zealand	−24·4
Proteaceae		
Banksia	Australia	−26·3
Honeydew	New Zealand	−25·9

TABLE 3. Summary of $\delta^{13}C$ values for all honey samples

	Number	*Mean*	*Range*	*s*	*c.v.*
US honey*	84	−25·2‰	−22·5‰ to −27·4‰	0·94‰	3·73%
Imported honey	35	−25·8	−23·9 to −27·4	0·97	3·76
All samples	119	−25·4	−22·5 to −27·4	0·98	3·86

* including 4 honeydew honeys

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